With the tools available in the early twentieth century, researchers were able to develop two types of information about mitochondria: (a) their shape and location in cells, and (b) their composition. Microscopic examination showed that the appearance of mitochondria is relatively stable across species, but varies in degree of elongation and thickness according to the cell type. Cowdry (1924) noted that in gland and nerve cells as well as embryonic cells, they were observed as filaments but that on injury, their shape changes, "providing by far the most delicate criterion of many types of cell injury at our disposal" (p. 317).

Evidence about the composition of mitochondria came primarily from reactions with various reagents. Their solubility with acetic acid, as well as with alcohol, ether, and chloroform, indicated a phospholipid constitution. The failure of mitochondria to stain with Sudan III indicated that they did not contain fat, and failure to stain with Millon's reagent indicated little if any protein.

Once dissociated from Altmann's conception of an elementary organism carrying out all basic metabolic processes, a natural question was what function mitochondria perform. Kingsbury (1913) noticed that the fixatives yielding the best visualization of mitochondria – osmic acid, potassium dichromate, and formalin – all depend on reducing substances. Picking up the thread from Michaelis, he advanced the proposal that mitochondria play a critical role in respiration.[20] Cowdry further noted that the amount of mitochondria in a cell was positively correlated with its level of activity (division, secretion, etc.) and negatively correlated with the amount of fat in it (an indication of decreased respiration):

> We have two lines of observation to harmonize: this association of abundant mitochondria with intense protoplasmic activity and a reciprocal relationship which appears to exist between the amount of mitochondria and the amount of fat. Where there are few mitochondria there is often much fat, and vice versa. Decreased oxidation favors the deposition of fat and increased oxidation hastens its elimination, which suggests at once the existence of some connection between the amount of mitochondria and the rate of oxidation; and their abundance in the more active stage of the life of the cell, when protoplasmic respiration is rapid, points to the same tentative conclusion. (Cowdry, 1924, p. 321)

[20] According to Bourne (1962, pp. 70–1), Kingsbury's major evidence was that "anesthetics such as ether or chloroform, which depressed cellular respiration and the respiration of the animal in general, also broke up mitochondria in the cell."